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in Transgenic Tobacco Plants

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The *Agrobacterium rhizogenes* T-DNA oncogene *rolD* under the control of its own 5′ regulatory region was transferred to day-neutral tobacco plants. The main trait induced by *rolD* in transgenic plants is a striking precocity in flower setting and a strong enhancement of the flowering potential. In *rolD* plants, early flowering is followed by the very rapid growth of numerous lateral inflorescences. The analysis of several morphological and histological parameters suggests that some characteristic morphological abnormalities observed in *rolD* plants can be accounted for by their early reproductive phase transition and points to the involvement in the transition of a greater portion of the plant body than is the case for untransformed tobacco. The *in vitro* morphogenic potential of tissues from *rolD* plants was also tested. Superficial thin cell layer explants from *rolD* plants show an earlier and much enhanced flower organogenesis, compared to controls, both on flowering and on hormone-free medium.

INTRODUCTION

Transfer of T-DNA from the Ri (root-inducing) plasmid of *Agrobacterium rhizogenes* to plant cells is responsible for the neoplastic growth of hairy roots at the site of bacterial infection on dicotyledonous plants. Hairy roots can be regenerated into plants containing the Ri T-DNA and showing a complex set of more or less pronounced morphological and developmental aberrations, known as the hairy root phenotype. The agropine type Ri T-DNA contains 18 open reading frames (ORF), 4 of which, ORFs 10, 11, 12, and 15, coincide with genetic loci identified by transposon-insertion mutagenesis. Inactivation of each of these 4 loci causes a distinct change in hairy root induction and they were consequently denominated *rol* (root loci) *A*, *B*, *C*, and, *D* respectively (for a recent review, see Costantino *et al.*, 1994). Transfer of ORFs and *rol* genes to plants (Cardarelli *et al.*, 1987; Spena *et al.*, 1987; Schmulling *et al.*, 1988; Sinkar *et al.*, 1988) allowed analysis of the individual and of the partly synergistic effects of *rol* genes *A*, *B*, and *C*. Thus, wrinkled leaves were shown to be due to *rolA* (Schmulling *et al.*, 1988; Sinkar *et al.*, 1988), reduced apical dominance to *rolC* (Schmulling *et al.*, 1988), while adventitious rooting and branched and plagiotropic roots are characteristic effects of *rolB* (Cardarelli *et al.*, 1987; Spena *et al.*, 1987; Schmulling *et al.*, 1988). Overexpression in transgenic plants of another Ri T-DNA-borne gene, ORF13, causes a number of phenotypic alterations mediated by a diffusible factor(s) (Hansen *et al.*, 1993). In addition, recombinant *Agrobacterium* strains harboring the *rolA*, *B*, or *C* genes have been found capable of triggering—with different efficiency ($B \gg A > C$)—the formation of neoplastic roots upon infection of different plant tissues (Cardarelli *et al.*, 1987; Spena *et al.*, 1987; Capone *et al.*, 1989).

The bacterial *rol* genes can thus act as plant oncogenes capable of affecting plant growth and development. Biochemical analysis relates two *rol* genes to plant growth factors. The gene *rolC* has been shown to be capable of hydrolizing cytokinin glucosides (Estruch *et al.*, 1991a) and *rolB* has been implicated in altering auxin perception and/or concentration (Spanò *et al.*, 1988; Estruch *et al.*, 1991b; Maurel *et al.*, 1991). However, the mechanism of action of the *rol* oncogenes within the plant is still not understood (Costantino *et al.*, 1994).

In the case of *rolB*, it has been recently shown that its primary action in morphogenesis, at least *in vitro*, consists in strongly promoting the formation of meristems (Altamura *et al.*, 1994), the morphogenic structures that deter-

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mine the differentiation of plant organs (Steeves and Sussex, 1989).

So far, all of the work on the *rol* plant oncogenes has been focused on *rolB*, *rolC*, and, to a lesser extent, *rolA*. In contrast, despite its early recognition as a root locus (White *et al.*, 1985), no further work has been carried out on *rolD*. Here, we describe the striking effect of this gene in stimulating flower setting of day-neutral tobacco plants and the consequences on the morphology and histology of these plants and on the morphogenic potential of their tissues.

MATERIALS AND METHODS

Bacterial Strains and Media

Agrobacterium strains were grown at 28°C in yeast mannitol broth (YMB). *Escherichia coli* strains were grown in Luria broth (LB) at 37°C. Media for strains harboring pBin19 and constructs thereof were supplemented with 100 μg/ml kanamycin (km) (YMB) or 30 μg/ml km (LB).

Plasmid Constructs

A 1967-bp DNA segment encompassing the whole of the *rolD* coding sequence and a 5′ noncoding region of 587 bp were obtained by digestion of the K13 clone of the pRi1855 TL-DNA (Capone *et al.*, 1989) with *Dra*I. This DNA was cloned in pBin19 (Bevan, 1984) and subsequently transferred to *Agrobacterium* LBA4404 by standard techniques.

Tobacco Stem Infections

Nicotiana tabacum L. c.v. Petite Havana SR1 (Maliga *et al.*, 1973) plants were grown aseptically on MS medium (Murashige and Skoog, 1962), at 25°C on a 16-hr illumination period. Stems of 1-month-old plants were punctured with a toothpick dipped in a culture of *Agrobacterium* harboring either the pBin19–*rolD* construct or pBin19 only. Plants were scored for root growth at the wound sites after 10 days and adventitious root growth was followed for up to 1 month.

Transgenic Plants

Transgenic plants (T1) were obtained by leaf-disc infections of SR1 plants and selected on 300 μg/ml km. Regenerants were initially grown *in vitro* at 25°C under a 16-hr illumination period in MS medium and then transferred into an illuminated (16-hr illumination period), thermostated (20–24°C) greenhouse and manually self-fertilized. For the analysis of T2 and T3 plants, seeds were germinated on MS medium, selected on 300 μg/ml km, and grown as described for T1.

PCR Analysis

Two 21-bp synthetic oligonucleotides (5′ ATGGCCAAACAACTTTGCGAG 3′ and 5′ ATGCCCGTGTTCCATCGGGCC 3′), homologous to the terminal sequence of the 5′ and 3′ ends of the *rolD* coding region, were utilized for assessing the presence of *rolD* in the DNA of T1, T2, and T3 plants by PCR analysis. Thermal cycles for amplification were 1 min at 95°C, 2 min at 60°C, and 3 min at 72°C (25 cycles).

Explants and in Vitro Culture

Thin cell layers (TCL) were excised from the rachis of the inflorescence (full flowering stage) and from the stem region (fourth or fifth node counting from the lowest axillary inflorescence) of various clones of *rolD* plants and of untransformed SR1. Rachis and stem internodes were surface-sterilized for 10 min with a 10% dilution of commercial bleach (5.6% active Cl) and rinsed three times for 10 min in sterile distilled water. TCLs (2 × 10 mm), composed of epidermis, subepidermal chlorenchyma, and cortical parenchyma, were excised aseptically and cultured in flowering medium (rachis explants, 1 μM indole-3-acetic acid, 1 μM kinetin, Tran Thanh Van *et al.*, 1974), or hormone-free medium (rachis explants), or rooting medium (stem explants, 10 μM indolebutyric acid, 0.1 μM kinetin, Tran Thanh Van *et al.*, 1974); the pH was adjusted to 5.6 with NaOH and 0.8% agar was added to the media.

The explants were cultured for 30 days at 26°C under a 16-hr illumination period (flowering and hormone-free media) or in the dark (rooting medium). Cultures were repeated twice with similar results. The significance of differences between means and of differences between percentages was evaluated by the Student's test and, respectively, by the χ^2 test. Inflorescences were counted as single flowers.

Histological Analysis

Leaves, bracts, and terminal and axillary buds from NtD-26 and NtD-30 clones of *rolD* plants and from SR1 controls were fixed in FAA (70% ethanol:glacial acetic acid:formalin, 18:1:1), dehydrated, and embedded in paraffin (melting point 52°–54°C, Merck). Thin (5-μm) sections were stained with eosin and Carazzi's hemalum (Mazzi, 1977).

RESULTS

Cloning of rolD

The gene *rolD* was isolated from clone K13 encompassing the suitable segment of the T-DNA of *A. rhizogenes* Ri plasmid pRi1855 (Capone *et al.*, 1989). The whole of the *rolD* coding sequence and 587 bp at its 5′ were cloned in the plant vector pBin19. Based on the sequence of the T-DNA (Slightom *et al.*, 1986), *rolD* is preceeded by ORF16 on the same coding strand and the distance between the stop codon of the latter and the start codon of the former is 189 bp. Thus, the cloned 5′ noncoding region of *rolD* actually encompasses 398 bp of ORF16. However, no transcript has been assigned to ORF16 (Durand-Tardif *et al.*, 1985) and this latter should therefore be regarded as noncoding. The putative translation product of *rolD*-amino-acid is a 344 (aa)-long protein with no obvious peculiarity in its aa composition. Comparison with protein sequences (SwissProt, Pir, Kabatp non-redundant data base) and with the possible translation products of nucleotide sequences (GenEMBL, XEMBL, Kabatn non-redundant data base) did not show any significant sequence homology. A search in the Prosite col-

lection revealed no recognizable motifs or domains in the sequence of RolD.

Tobacco Stem Infections with rolD

As *rolD* had been originally identified as a ''root locus'' (White *et al.*, 1985), we tested the effect of infections on plants with an *Agrobacterium* strain carrying the above-described *rolD* construct. Thus, stems of 10 Petite Havana SR1 tobacco plants were punctured and inoculated with such a strain and 10 more with an *Agrobacterium* harboring the vector plasmid alone. Wound sites were scored for root production after 10 days. One or two adventitious roots were produced around the wounds of about half of the plants inoculated with the *rolD*-harboring strain but also of plants inoculated with the control strain. The inoculations were repeated three times (for a total of 30 plants for each type of inoculation) with analogous results, i.e., no difference in adventitious root production or in root morphology and growth pattern between the *rolD*- and mock-infected plants. Thus, in contrast with *rolB* and, to a lesser extent *rolC* and *rolA* (Cardarelli *et al.*, 1987; Spena *et al.*, 1987), the root locus *rolD* is not capable per se of root induction.

Early Flowering of rolD plants

Leaf discs from SR1 plants were infected with the *Agrobacterium* strains harboring the *rolD* construct or the vector plasmid alone. Seven different *rolD* transformants—T1 plants, clones NtD-25, -26, -29, -30, -31, -33, and -34, all shown to contain *rolD* by PCR analysis (not shown)—and 10 BIN19 regenerants as well as 5 untransformed SR1 plants were transferred to the greenhouse.

All *rolD* plants lost the rosette habit during the first week after transfer to the greenhouse, compared to the 3 to 4 weeks necessary for the bolting of BIN19 and SR1 plants (from now on both regarded as controls). The rosette leaves of the *rolD* plants are similar in shape to those of the controls. However, after the early bolting of the stem, the leaves of the former remained smaller and with characteristically curved, pointed tips. No differences in apical dominance were observed between rolD and control plants.

After 15 days in the greenhouse, some of the *rolD* plants began to flower and by 45 days all of them reached anthesis (*i.e.*, the time of maturation of the sexual organs in the flower). At anthesis of the first flowers, *rolD* plants presented an average of 10–11 internodes and a height of about 30 cm. In contrast, at the 10–11 internode stage, control plants were all still vegetative and anthesis of the first flowers was not observed until at least 90 days after transfer to the greenhouse, when plants reached, under our greenhouse conditions, the expected developmental stage of 22 to 28 internodes and a height of about 50 cm. Figure 1 shows a flowering *rolD* plant (right) and a vegetative control (left) at the 10–11 internode stage.

Floral Phenotype of rolD Plants

In order to confirm these observations, the growth of five different plants of each of the two transformants NtD-26 and NtD-30 was followed. Both NtD-26 and NtD-30 were shown by DNA gel blot to contain one copy of *rolD* in their genome (not shown). The same developmental pattern of *rolD* plants described above was observed again.

Morphological parameters of the 10 transgenic and of 15 control plants were measured at anthesis, and the results subjected to statistical analysis as reported in Table 1. The results of a comparative histological analysis are reported in Table 2.

In summary, in leaves we found effects on both size and the extent of tissue layers (compare Figs. 2A and 2B). In flowers, we observed overall effects on organ size, but no change in organ arrangement.

The most striking effect of *rolD* is in the percentage of axillary buds that show floral character. Histological sections revealed an average of five axillary buds of floral (inflorescence) type, compared to an average of two in controls. Figure 2 shows the lateral inflorescence at the fifth axil of a *rolD* plant (Figs. 2C and 2D) compared to the vegetative bud at the same axil of a control plant (Fig. 2E). Below the axillary inflorescence buds, two multiple vegetative buds are present in *rolD* plants, compared to only one in control plants. The numerous axillary inflorescences of *rolD* plants develop very quickly and their rachis grows unusually fast (sylleptic inflorescences). Figure 3 shows a *rolD* plant with sylleptic axillary inflorescences (right), compared to a flowering control plant (left).

The root systems of *rolD* and control plants were also compared, but no significant differences were observed.

Stability of the Floral Phenotype in T2 and T3 Generations

Despite the heterostyly (style protrusion) of their flowers which prevents the spontaneous production of seed capsules, T1 *rolD* plants could be manually selfed and yielded normally viable seeds. Seeds from four different transformant clones, NtD-25, 26, 30, and 33, were selected on kanamycin and 51 T2 plants (15 each of NtD-25, 26 and 33 and 6 of NtD-30) were scored. One-third (17 of 51) of the T2 plants showed the early flowering and the overall floral phenotype described in the previous paragraph for the T1 parentals. Another third (17 plants) showed a precocious flower induction, at a stage of around 15 internodes, but the maturation of the flower buds was slower and anthesis was reached as late as and when plants were as tall as and with as many internodes as SR1 controls.

Early flowering T2 NtD-26 and T2 NtD-30 plants were then selfed and T3 progeny obtained after selection on kanamycin; 15 plants of each type were scored. One-third of the T3 plants (10 of 30) showed the early flowering and overall floral phenotype described in detail for the T1 plants and observed also in a fraction of the T2 progeny. Another 11 T3 plants showed the early induction but normal anthesis time described above for an equivalent fraction of the T2 progeny.

FIG. 1. A vegetative untransformed SR1 plant (left) and a flowering *rolD* plant (right) at the stage of 10–11 internodes.
FIG. 3. A flowering untransformed SR1 plant (left) and a flowering *rolD* plant after the development of sylleptic axillary inflorescences (right).

In Vitro Morphogenesis of rolD Explants

TCL explants were excised from NtD-26, NtD-30, and SR1 plants and cultured *in vitro* under hormonal conditions of flower or root morphogenesis and on hormone-free medium (see Materials and Methods). As reported in Table 3, on both flowering and hormone-free media *rolD TCL*s show a flowering response significantly enhanced compared to controls. On both media, in fact, the percentage of *rolD* TCLs with flowers and the number of *rolD* explants producing only flowers and no vegetative buds are both much higher than in controls. The average number of flowers on each *rolD* explant is also much higher, the more so as transformed explants produce mostly inflorescences—often geminate and with an average of four to five flowers each—which are counted as single flowers in Table 3.

In addition, flowers appear much earlier in *rolD* TCLs than in controls. By Day 8 of culture on flowering medium, flowers are present on about 30% of *rolD* explants and on none of the controls. At Day 12, still only 8% of control TCLs show flowers, compared to 47% of *rolD* explants. Moreover, in contrast with flowers from untransformed TCLs, flowers from *rolD* explants show a fast and vigorous growth and are capable of anthesis *in vitro* even in hormone-free medium.

In contrast with the strong enhancement and precocity of the flowering program, root morphogenesis appears depressed in *rolD TCL*s, as reported in Table 3. Upon culture on rooting medium, in fact, the percentage of *rolD* explants with roots and the number of roots per explant are reduced compared to the controls and the percentage of *rolD* explants producing flowers is higher.

DISCUSSION

In this paper we describe and analyze the striking effect of the plant oncogene *rolD* in accelerating induction of the flowering process and in enhancing flower formation in tobacco.

The developmental stage at which *rolD* plants flower is very precocious, as they show in average of 10–11 internodes compared to the 22–28 internodes of untransformed SR1 plants. In day-neutral tobacco, such as the one analyzed in this work, the number of nodes produced before

TABLE 1

Phenotypes of Flowering *rolD* and Control Plants

	Average ± SE (mm)		
	rolD	Control	sl
Plant height	311.7 ± 2.0 (n = 10)	513.5 ± 2.2 (n = 15)	* *
Left blade length	59.9 ± 1.2 (n = 68)	89.5 ± 2.0 (n = 44)	* *
Left blade width	21.6 ± 0.5 (n = 68)	33.3 ± 0.8 (n = 44)	* *
Petiole length	9.3 ± 0.3 (n = 68)	12.6 ± 0.5 (n = 44)	* *
Petiole width	2.0 ± 0.1 (n = 68)	3.0 ± 0.1 (n = 44)	* *
Bract blade length	23.0 ± 2.2 (n = 9)	42.9 ± 4.3 (n = 7)	* *
Bract blade width	4.4 ± 0.7 (n = 9)	14.0 ± 2.1 (n = 7)	* *
Corolla length	30.2 ± 0.4 (n = 10)	38.7 ± 0.6 (n = 10)	* *
Style protrusion	8.0 ± 0.5 (n = 10)	0 (n = 10)	

Note. sl, significance level.
** $P < 0.01$.

the onset of flowering is precisely regulated, and the shoot meristem originates a defined number of leaves before reproductive transition takes place (Mc Daniel and Hsu, 1976; Mc Daniel, 1978). The root–flower positional effect, i.e., the minimal number of nodes that normally separates the root system from the shoot meristem and which determines the developmental stage for flowering (Mc Daniel, 1980), is thus perturbed in *rolD* plants. On average, at anthesis of the first flowers, five of the axillary buds of *rolD* plants are of the floral type while in normal SR1 only two axillary meristems have undergone reproductive transition. In addition, flowering *rolD* plants have twice as many multiple axillary vegetative buds—a marker of flower transition (Tian and Marcotrigiano, 1994)—as normal SR1. If the reduced number of nodes of *rolD* plants is taken into account, it comes into view that a great part of the *rolD* plant (5 + 2 nodes of 10–11) is involved in the floral transition as opposed to untransformed controls, where the transition occurs only in the uppermost portion of the plant (2 + 1 nodes of 22–28).

The numerous floral axillary buds of *rolD* plants develop quickly into fast-growing inflorescences. In plants with terminal inflorescence, such as tobacco, the development of axillary meristems is an ''almost universal event of the floral transition'' (Bernier, 1988), which appears amplified in *rolD* plants.

In contrast with the other *rol* oncogenes, each of which alters in different ways the development of transgenic plants (Cardarelli *et al.*, 1987; Spena *et al.*, 1987; Sinkar *et al.*, 1988), *rolD* does not seem to induce significant morphological modifications other than those possibly amenable to a very precocious—and diffused to most of the plant—transition to the reproductive phase. The most conspicuous alteration of *rolD* plants is in the leaves, which are smaller and thinner than normal. As ''the last leaves before the reproductive structures are generally of small size and simple shape'' (Bernier, 1988), this is a further indication that in *rolD* plants most of the plant body participates in the reproductive transition.

The effect of *rolD* on flowering is stably transmitted in a substantial fraction of the progeny. At least up to the T3 generation, one-third of the plants show in fact the same

TABLE 2

Histological Features of Leaves[a] and Bracts from *rolD* and Control Plants

	Average ± SE		
	rolD	control	sl
Thickness of the leaf (μm)	120.0 ± 2.8 (n = 44)	167.0 ± 5.7 (n = 18)	* *
Height[b] of upper epidermal cells (μm)	15.5 ± 0.5 (n = 125)	16.5 ± 0.5 (n = 100)	ns
Height[b] of lower epidermal cells (μm)	10.3 ± 0.3 (n = 102)	10.3 ± 0.3 (n = 117)	ns
Height[b] of the palisade cells (μm)	29.0 ± 0.5 (n = 191)	44.9 ± 0.6 (n = 191)	* *
Intercellular spaces in the spongy (%)	35.3 ± 1.1 (n = 35)	43.2 ± 1.3 (n = 60)	* *
Leaf stomatal number/mm^2 (upper epidermis)	22.8 ± 3.4 (n = 23)	36.9 ± 3.7 (n = 21)	*
Leaf stomatal number/mm^2 (lower epidermis)	133.0 ± 11.3 (n = 23)	111.0 ± 6.4 (n = 21)	ns
Thickness of the bract (μm)	124.0 ± 2.2 (n = 26)	102.0 ± 3.9 (n = 8)	* *
Bract stomatal number/mm^2 (upper epidermis)	41.4 ± 3.0 (n = 23)	38.9 ± 3.4 (n = 21)	ns
Bract stomatal number/mm^2 (lower epidermis)	194.4 ± 15.0 (n = 23)	177.0 ± 5.9 (n = 21)	ns

Note. sl, significance level; ns, not significant.
[a] From the median region of the fifth to ninth cauline leaves.
[b] In transection.
* $P < 0.05$.
** $P < 0.01$.

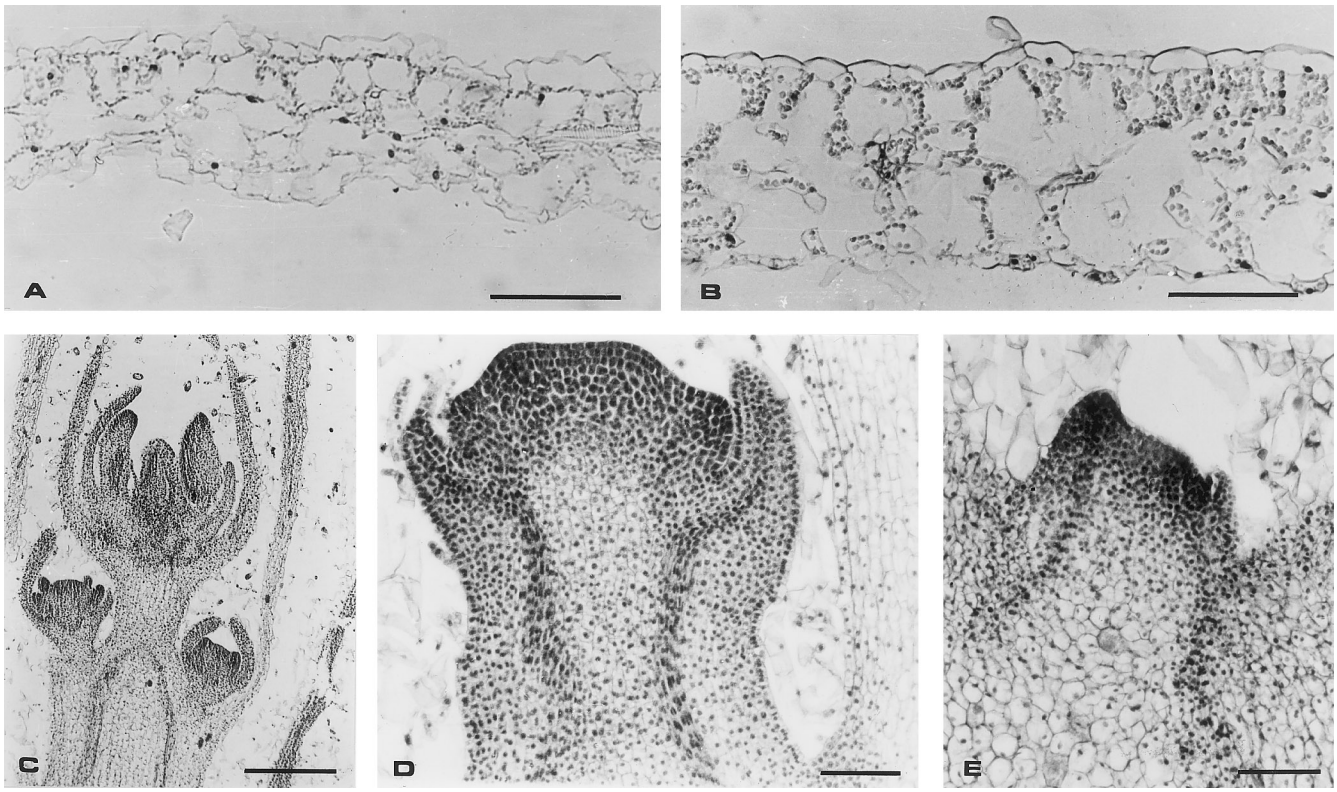

FIG. 2. Histological transections of (A) a leaf blade of a *rolD* plant (bar, 100 μm); (B) a leaf blade of an untransformed SR1 plant (bar, 100 μm); (C) a lateral inflorescence at the fifth axil of a *rolD* plant (bar, 0.5 mm); (D) a developed axillary flower of the lateral inflorescence shown in C (bar, 100 μm); and (E) a vegetative axillary bud at the fifth axil of an untransformed SR1 plant (bar, 100 μm). All sections are from flowering plants.

early flowering and overall morphology as the T1 transformants.

The specificity of the effect of *rolD* can be best assessed *in vitro* from the morphogenic potential of superficial explants from *rolD* plants. In contrast with *rolB,* which stimulates in TCLs the production of all types of organs—roots as well as flowers and vegetative shoots (Altamura *et al.*, 1994, 1995)—*rolD* stimulates specifically the production of flowers. Under culture conditions for flower induction, flowering response in *rolD* TCLs is much earlier and more abundant than that in controls. Furthermore, flowering is very pronounced even in hormone-free medium, indicating that *rolD* tissues are intrinsically highly committed to this type of organogenesis. In contrast, production of vegetative shoots is unaffected and root organogenesis is somewhat depressed by *rolD,* as transformed TCLs also tend to produce flowers under rooting conditions. In whole plants, no significant effect of *rolD* on the root system was observed.

It is difficult to speculate at this stage on the molecular mechanism through which *rolD* may exert its effect on flowering. In day-neutral plants, floral induction is mainly under the control of an endogenous factor(s). As to the nature of such a factor(s), little is firmly established. After decades invested in the search for a single elusive ''florigen'' factor and in the characterization of the effects of nutrients and plant hormones on the promotion or inhibition of flowering, a multifactorial model where both assimilates and hormones play a role in the control of this process has been proposed (Bernier, 1988; Bernier *et al.*, 1993). Within this hypothesis, a suggestion for a possible biochemical function of *rolD* may come from the general strategy of *Agrobacteria* in controlling the growth of transformed plant cells and from the functions of other Ri plasmid-borne oncogenes. Crown gall tumors induced by *Agrobacterium tumefaciens* grow off the plant hormones (auxin and cytokinin) synthesized by T-DNA-encoded enzymes. As for *A. rhizogenes,* the proteins encoded by *rolC* and *rolB* have been shown to be able to hydrolize sugar conjugates of either plant growth regulators or structurally related molecules (Estruch *et al.*, 1991a,b). Thus, the protein encoded by *rolD* might exert its effect on flowering by altering the concentration of plant hormones. As far as the role of these latter in flowering, most of the work has been devoted to photoperiodic plants (i.e., plants requiring long or short days to flower). In general, cytokinins have been shown to have a promotive effect on flower induction in long-day plants and to be inhibitory on short-day ones, though contrasting results have been often reported (for a review, see Reid *et al.*,

TABLE 3
In Vitro Organogenesis of Explants (TCL) from *rolD* and Control Plants

	rolD	Control	sl
FM			
TCLs with flowers	72.3% (n = 65)	38.5% (n = 39)	*
TCLs with flowers only	50.8% (n = 65)	23.1% (n = 39)	*
No. of flowers[a] per TCL	10.0 ± 1.4 (n = 65)[b]	2.9 ± 0.6 (n = 39)[b]	*
HF			
TCLs with flowers	57.7% (n = 78)	26.7% (n = 30)	*
TCLs with flowers only	34.6% (n = 78)	10.0% (n = 30)	*
No. of flowers[a] per TCL	7.2 ± 0.5 (n = 78)[b]	4.5 ± 1.1 (n = 30)[b]	*
TCLs with roots	23.4% (n = 77)	79.1% (n = 86)	*
	(1.3 ± 0.1)[c]	(2.5 ± 0.2)[c]	*
RM			
TCLs with vegetative buds	59.7% (n = 77)	57.0% (n = 86)	ns
TCLs with flowers	16.9% (n = 77)	6.0% (n = 86)	*

Note. FM, flowering medium; explants from inflorescence rachis; HF, hormone-free medium; explants from inflorescence rachis; RM, rooting medium; explants from the middle region of the stem; sl, significance level; ns, not significant.
[a] Inflorescences counted as single flowers.
[b] Average ± SE.
[c] Average number of roots per explant ± SE.
* $P < 0.05$.
** $P < 0.01$.

1991). High levels of auxin seem, at least in short-day plants, to exert an inhibitory effect, but low levels are required for floral transition (for a review, see Bernier, 1988). The most extensively investigated plant growth regulators in relation to flower induction are gibberellins, the promotive effect of which depends on the structure and concentration of the particular member of this complex family of hormones (Reid *et al.*, 1991). Stimulation of flowering in some day-neutral plants by gibberellins has also been reported (Kinet, 1993). The evidence available so far on the role of hormones in flower induction is thus still too fragmentary and controversial to be of help in directing the search for a hormone-related biochemical role of the *rolD* oncogene.

In future work, it will be important to assess whether *rolD* also stimulates flowering in photoperiodic plants and, in particular, in the model species *Arabidopsis thaliana*, where a systematic effort to identify the genetic determinants of flowering is being carried out. Genes specifying flower meristem identity have been identified—*LEAFY*, *APETALA*, and *CAULIFLOWER* (Yanofski, 1995, and refs. therein; Weigel and Nilsson, 1995)—and it will be interesting to analyze whether and how *rolD* would affect the timing and pattern of their expression. Several early- and late-flowering *Arabidopsis* mutants have also been isolated. The genes affected in two of the late-flowering mutants (*ga1* and *gai*) are involved in gibberellin biosynthesis and sensitivity (for a recent review, see Coupland, 1995). Complementation of these or other late-flowering mutations by expression of the oncogene *rolD* could open exciting perspectives in the analysis of the mechanisms of floral induction.

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